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'Klebsiella' Densities in Waters Receiving Wood Pulp Effluents

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16. ABSTRACT

Surface waters receiving pulp mill effluents were examined for the presence of total coliforms, fecal coliforms, and Salmonella species. Fecal coliforms were biochemically identified as belonging to the Escherichia, Klebsiella or Enterobacter genera. Sixty percent of the isolates were identified as Klebsiella, 34% were found to be Escherichia coli and the remaining 6% were primarily Enterobacter species.

The high densities of fecal coliforms, a majority of which were Klebsiella species, in the almost complete absence of Salmonella was attributed to multiplication in the carbohydrate rich aquatic environment. The findings show a need for a more specific indicator of the wastes of warm-blooded animals.

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The density of coliform bacteria in estuarine and fresh waters has been used for some time as an index of contamination with the fecal wastes from warm-blooded animals and, therefore, for the presence of possible enteropathogenic microorganisms contained therein. The validity of using all coliform bacteria as indicators of fecal pollution has been questioned because some coliform biotypes have been isolated from soils and water not subject to recent fecal pollution;[1] some coliform biotypes have been reported to multiply in the aquatic environment when nutrient levels and temperatures are adequate;[2] and the die-off of coliforms in the aquatic environment may not parallel that of enteric pathogens.[3-5] The enumeration of only that portion of the coliform population defined as "fecal" (*aerogenesis* in EC broth incubated at 44.5° ± 0.2°C) was proposed as a means of circumventing the first two objections.[6] Several workers have reported that the majority of coliforms isolated from the feces of warm-blooded animals and from solids and water recently contaminated with fecal material could be classified as fecal and that fecal coliforms represent a relatively small percentage of the coliforms in "uncontaminated" soils and water.[7, 8] Also, "regrowth" was considered to occur only among nonfecal coliforms.[9] Recent evidence suggests, however, that certain biotypes of the genus *Klebsiella*, which meet the criterion for fecal coliforms, can multiply in waters contaminated with the wastes from wood pulping processes.[10] The present study was designed to confirm the presence of large numbers of *Klebsiella* organisms in waters receiving pulp mill effluents.

Materials and Methods

Study area. The study area is located near the northwest tip of Mobile Bay adjacent to the mouth of the Mobile River (Figure 1). Seagoing cargo ships enter the river at this point for destinations upriver. Approximately 1,800 m to the south, a 2,600-m channel (Arlington) has been dredged for ship passage into a dock area and turning basin now used by U. S. Coast Guard vessels. Northward, a second channel (Garrows Bend), about 2,300 m in length, runs from the turning basin to a dirtfill bridge. This channel receives the effluent from the pulp mill. The sampling stations were located near the junction of the two channels as shown in Figure 1. Stations 1 through 7 form a transect across the channel about 46 m to the southeast of the pulp mill effluent outfall. At Station 8, the remainder of an old pier serves as a roost for gulls and pelicans. An old sunken ship mast is located at Station 10. Northeast and southwest of the pulp mill, two drainage ditches form part of a storm sewer system designed to carry waters from heavy rains to the bay area. In the absence of heavy rainfall, there was little water in the ditches, and that which was there appeared to be stagnant and very dark in color. There was an odor of diesel fuel in the northeast ditch. Samples of water taken from that ditch contained many black particles. The water from the southwest ditch (contained in small stagnant pools) was relatively clear.

During tidal cycles or fresh winds, there is some degree of water interchange between Mobile Bay and Arlington Channel. A flow rate study in the Garrows Bend channel, conducted during a high to falling

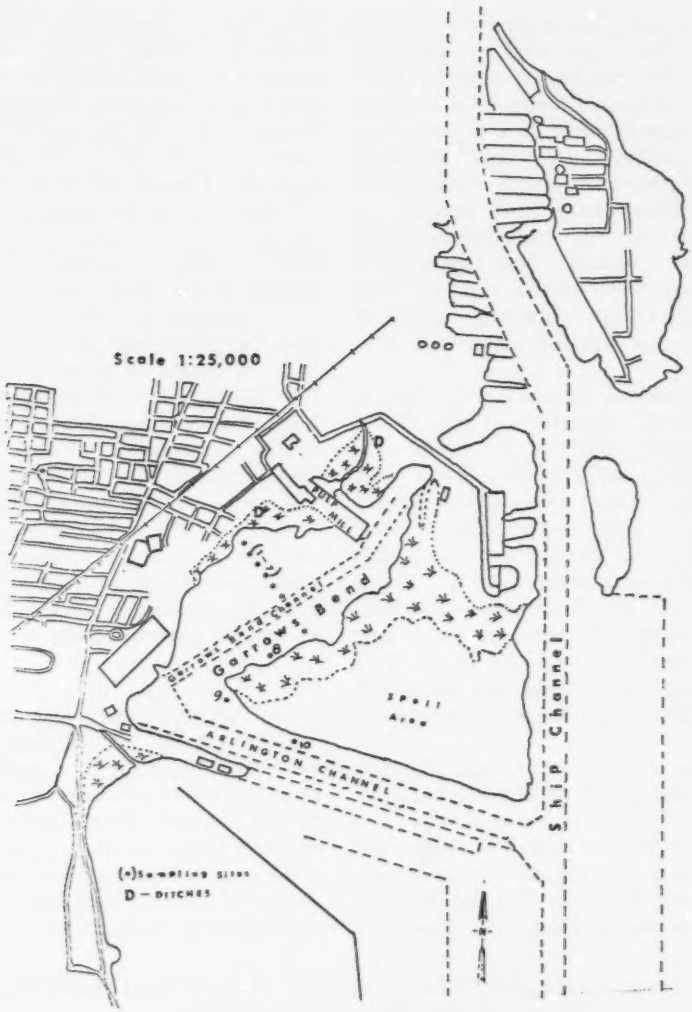

FIGURE 1.—Location of sampling stations below pulp mill on Garrows Bend off the northwest tip of Mobile Bay, Ala.

tide, indicated very little surface interchange between the waters in Garrows Bend and the ship channel waters, although strong northeast winds did cause relatively rapid movement of the drogues toward the turning basin. It cannot be stated unequivocally that there was no domestic waste entering the area. Sources of fecal pollution of the magnitude required to account for the fecal coliform densities observed in the present study were not detected, however.

Sample collection. Samples were collected five times during the study period, which extended from late November through early June. Gauze traps [11] were prepared from eight-ply unbleached muslin measuring 10 × 30 cm so that each trap could be easily separated into two four-ply strands for assay after exposure. The traps

were foil wrapped before sterilization in an autoclave. One gauze trap was placed at each of the 10 sampling sites in the study area so that it was submerged 20 to 25 cm below the water surface at low tide. The traps were retrieved from the water after 72 hr and placed in sterile "twirl pack" bags. A surface water grab sample was collected at each site immediately before the trap was retrieved. The traps and the water samples were placed in an ice chest for transport to the laboratory. Assay of the samples was initiated within 4 hr after collection.

Assay. One-half of each trap (one of the two four-ply strands) was placed in a flask containing 500 ml of tetrathionate broth for the isolation of *Salmonella*. Bismuth sulfite, xylose lysine desoxycholate, and brilliant green agar plates [12] were streaked from the tetrathionate broth after 24 and 48 hr incubation at 35°C. Suspicious colonies from these agar plates were picked to triple sugar iron and urea agar slants incubated for 18 to 24 hr at 35°C; those isolates which indicated *Salmonella* were identified according to Edwards and Ewing.[12]

The other half of the trap was placed in a flask containing 500 ml of phosphate buffered water.[11] The flask was shaken for 5 min on a mechanical shaker at 60 strokes/min to dislodge the organisms from the trap into the buffered water. The resultant suspensions and the surface water grab samples were examined by five-tube, most probable number (MPN) assays carried through the presumptive test for coliforms and into EC medium to test for fecal coliforms.[11] The EC gas-positive tubes then were streaked onto eosin-methylene blue agar plates incubated 18 to 24 hr at 35°C (± 0.5°C). Representative typical and atypical colonies were picked from each plate to tubes of lactose broth, each containing a gas vial. After incubation at 35°C, gas-positive cultures were inoculated into EC broth and incubated in a water bath overnight at 44.5° (± 0.2°C). All EC gas-positive cultures then were inoculated into the appropriate media for identification according to Edwards and Ewing.[12] The tests used were the utilization of citrate and malonate; the production of indole, urease, and H_2S (in triple sugar iron agar); motility (motility test medium) and the methyl red, Voges-Proskauer; and the arginine dihydrolase, ornithine decarboxylase, and lysine decarboxylase.

RESULTS

The medium total and fecal coliform densities at the 10 sampling stations are presented in Table I. Large numbers of total and fecal coliforms were obtained at all the Garrows Bend stations. The total coliform:fecal coliform ratios generally were high, which suggests either a distant source of sanitary pollution, runoff, or the multiplication of a portion of the coliform

TABLE I.—Median MPN Values and Ranges for Coliform and Fecal Coliform Organisms from Water Supplies

Station	Total Coliform MPN/100 ml*		Fecal Coliform MPN/100 ml	
	Median	Range	Median	Range
1	790,000	70,000– 7,900,000	22,000	2,300– 79,000
2	490,000	33,000– 7,900,000	170,000	3,300–490,000
3	1,300,000	33,000– 7,900,000	18,000	2,300–300,000
4	1,600,000	140,000– 7,900,000	9,500	7,000–490,000
5	230,000	79,000–>1,600,000	17,000	3,300–170,000
6	4,900,000	490,000– 7,900,000	2,800	1,700– 49,000
7	330,000	46,000– 7,900,000	1,800	1,200– 33,000
8	230,000	23,000–>1,600,000	4,600	1,800– 70,000
9	130,000	7,900– 4,900,000	3,300	1,100– 49,000
10	7,900	4,900– 23,000	490	45– 4,900

* Presumptive test.

TABLE II.—Coliforms Isolated from EC Positive Tubes (Stations 1–7)

Determination	Nov. 30	Number of Isolates from Samples Collected on				Total	%
		Dec. 28	Feb. 24	Apr. 12	Jun. 7		
Total coliform MPN*	7,900,000	790,000	7,900,000	1,300,000	1,100,000		
Fecal coliform MPN*	140,000	79,000	130,000	79,000	490,000		
Klebsiella pneumoniae	36	177	112	90	114	556	60
Klebsiella sp.	10	53	21	27	19	130	
Enterobacter cloacae	0	1	1	26	0	28	5
Enterobacter aerogenes	14	9	9	0	0	32	
Escherichia coli	103	123	111	24	19	380	34
Other	1	0	8	1	0	10	1
Total						1,136	100
Temperature, °C	18.0	11.0	15.0	22.0	30.0		
pH	6.4	6.5	6.6	6.9	7.4		

* Median total or fecal coliform MPN/100 ml for Stations 1–7.

population under the influence of nutrients present in the water.

The nature of the fecal coliform population was examined by isolating and identifying the coliforms from the EC gas-positive tubes. The distribution of fecal coliform biotypes isolated from the water samples collected from Stations 1 through 7 during five sampling tours are presented in Table II. Included with these data are the total and fecal coliform MPN's and the temperature and pH of the samples. A total of 60 percent of the isolates were identified as *Klebsiella*, while 33.8 percent were found to be *Escherichia coli*. The remaining 6 percent were composed primarily of *Enterobacter* species, a value seemingly inconsistent with surface runoff as the source of the coliforms. The proportion of *E. coli* isolates seemed to decrease from February through June as the temperature and pH of the water increased. Although accurate estimates of the densities of the various biotypes in the samples could not be obtained, it may be safely assumed that the relative proportions of *Klebsiella* to *E. coli* in the samples were considerably greater than those reflected by the two-thirds to one-third isolation.

Although high fecal coliform recoveries were obtained from all of the gauze pad samples placed at the ten sampling stations

TABLE III.—Median MPN Values and Ranges for Coliform and Fecal Coliform Organisms from Gauze Traps

Station	Total Coliform MPN/100 ml		Fecal Coliform MPN/100 ml	
	Median	Range	Median	Range
1	2,300,000	330,000– 7,900,000	28,000	3,300–490,000
2	1,300,000	790,000– 7,900,000	130,000	7,900- 490,000
3	4,900,000	790,000–11,000,000	70,000	2,200–170,000
4	1,100,000	790,000– 7,900,000	330,000	22,000–700,000
5	1,100,000	490,000– 4,900,000	140,000	17,000–280,000
6*	11,000,000	330,000–13,000,000	7,900	2,200–220,000
7	2,300,000	1,300,000– 4,900,000	70,000	14,000–490,000
8	1,300,000	490,000– 3,300,000	33,000	11,000– 79,000
9	2,300,000	130,000– 4,900,000	23,000	4,900– 79,000
10	230,000	33,000– 460,000	2,300	2,200– 11,000

* Traps from February and January not recovered.

(Table III), only one *Salmonella* isolate was obtained during the entire course of the study, a group C *Salmonella* from Station 10.

DISCUSSION

The results obtained herein are consistent with those obtained by other workers. Duncan and Razzell,[10] working with isolates from wood bark samples taken from a paper mill logging pond, found that only a small percentage of the biotypes isolated were identified as *E. coli;* the majority were *Klebsiella* and *Enterobacter* species. Mallmann [13] examined the coliform biotypes in Lake Erie waters influenced by a river that received the discharge from a city's untreated primary effluent and three paper mills. He found that nearly 50 percent of the organisms producing gas in EC medium at 44.5°C for 24 hr were identified as *Aerobacter aerogenes.* The term *A. aerogenes* as used by some workers at that time included both *Enterobacter* and *Klebsiella* biotypes. Consequently, it cannot be determined which of these two genera accounted for the large percentage of EC gas-positive coliforms that Mallman found. Because *Enterobacter* species are infrequently EC gas-positive, it seems more likely that Mallmann's isolates were *Klebsiella* species as defined by present-day terminology. Thus, there is little doubt that species of the genus *Klebsiella* can multiply in the carbohydrate-rich environment provided by pulp mill effluents or the receiving waters for these wastes. These organisms are reported to have the extracellular enzymes necessary to use the complex carbohydrates found in some industrial effluents.[8]

The finding that *Klebsiella* species will multiply in carbohydrate-rich aquatic environments poses two problems. The first concerns the health hazard to individuals who use such waters as a source of recreation. Because at least some strains of *Klebsiella* are opportunists that are responsible for infections of the genitourinary and the upper respiratory tracts, the presence of inordinately large numbers of these organisms in recreational waters may be a health hazard. Epidemiological studies should provide an answer to this question.

A second consequence of the ability of *Klebsiella,* specifically those strains that are EC gas-positive ("fecal"), to multiply in carbohydrate-rich aquatic environments is the effect on the validity of the fecal coliform test as an indicator of the pollution of such waters with fecal wastes. With specific reference to the hazard of *Salmonella* infection, if salmonellae also multiply in these nutrient-rich waters, then the multiplication of the *Klebsiella* would necessarily not preclude the use of fecal coliforms as an indicator of the presence of salmonellae. In the present study such was not the case. The limited success in isolating *Salmonella* species is difficult to assess, however, especially because the use of gauze traps for the recovery of salmonellae has been reported by several workers [14, 15] and because these organisms have been recovered from waters in which the total and fecal coliform densities were quite low.[16] Possibly, the multiplication of the other organisms during the assay was so rapid as to overgrow the *Salmonella,* if they were indeed present in the study area waters.

The source of the *E. coli* found in the present study cannot be determined with any certainty. Mallmann [13] states that, in waters containing large amounts of organic matter, *E. coli* grows abundantly even in the absence of wastewater or bathing pollution. In the present study, however, *E. coli* was less frequently isolated during the summer months when the temperature was most conducive to the growth of this organism.

Those conditions of "nutrient pollution" that result in high densities of *Klebsiella* are not limited to the discharge of wood pulp effluents. Recently, Dufour *et al.*[17] reported high *Klebsiella* densities associated with the discharge of textile finishing plant effluents. Findings such as those described point out the need for care in interpreting total and even fecal data. Equally important, these findings show the need to increase the range of microbial and chemical measurements made, including a more

specific indicator of the wastes of warm-blooded animals.

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Authors. At the time that this paper was prepared, B. E. Huntley was chief of the Bacteriology Unit and A. C. Jones was a technician, Gulf Coast Water Supply Research Laboratory, Dauphin Island, Ala. Victor J. Cabelli was director, Recreational Water Quality Criteria Program, Water Supply Research Laboratory, National Environmental Research Center, Environmental Protection Agency, Cincinnati, Ohio. Requests for reprints should be made to Cabelli, who directs the Recreational Water Quality Criteria Program, National Marine Water Quality Laboratory, EPA, West Kingston, R. I. Cabelli is also associated with the Department of Microbiology, University of Rhode Island, West Kingston.

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